1.0 SCIENTIFIC ABSTRACT OF THE PROTOCOL

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease of unknown etiology, characterized by chronic joint swelling, pain, and stiffness leading to progressive damage and destruction of the joints. It is one of the most prevalent chronic inflammatory diseases in the US. More than two million Americans are affected, of whom over 75% are women, with the peak onset occurring between the ages 20 and 45. The incidence of RA has been estimated at 200,000 new cases every year.

TNF-α is strongly implicated as a major participant in the inflammatory cascade that leads to joint damage and destruction in RA patients. Although there is no cure, treatment has been revolutionized by the advent of anti-TNF-α therapies. These include etanercept (Enbrel®), infliximab (Remicade®) and adalimumab (Humira®), which consist of soluble TNF receptors, chimeric human-mouse anti-TNF-α monoclonal antibodies and fully human anti-TNF-α monoclonal antibodies, respectively. Clinical studies have shown these products to improve the signs and symptoms, inhibit the structural damage, and impact functional outcomes in patients with RA. However, some RA patients have one or two persistently symptomatic joints despite systemic TNF-α blockade, or only one or two problematic joints, which may demonstrate progressive joint destruction. These patients might benefit from intra-articular anti-TNF-α therapy using gene transfer, which provides a mechanism to achieve expression of therapeutic levels of TNFR:Fc protein in the joint without high levels in the systemic circulation.

tgAAC94 is a recombinant adeno-associated virus (AAV) serotype 2 vector genetically engineered to contain the cDNA for the human tumor necrosis factor receptor (TNFR)-immunoglobulin (IgG1) Fc fusion (hTNFR:Fc) gene. The DNA sequence of hTNFR:Fc in tgAAC94 codes for a protein identical to etanercept (Enbrel®). Intra-articular delivery of the hTNFR:Fc gene should result in expression of the secreted protein in the joint space and provide local therapeutic concentrations of soluble hTNFR:Fc at the site of disease with minimum exposure to the circulation, thus minimizing the potential for systemic adverse events.

AAV is a suitable vector for delivery of TNFR:Fc into joints. Extensive preclinical studies using several different transgenes in a variety of animal models have shown efficient and persistent gene transfer and expression without toxicity. Persistence of rAAV DNA

sequences in transduced host cells generally occurs by a mechanism involving transcriptionally active unintegrated episomal concatamers.

Nonclinical studies were conducted in three animal models: 1) normal rats, 2) rats with experimentally induced arthritis, and 3) non-human primates to support initiation of a Phase I safety study using tgAAC94. Since the expressed transgene protein product, human TNFR:Fc, is species-specific in its pharmacologic effect and results in an antibody response when administered to animals, a homologous vector, tgAAV-ratTNFR:Fc was developed for nonclinical testing in rats. The potential efficacy of the TNFR:Fc gene delivered by rAAV was demonstrated in a study of tgAAV-ratTNFR:Fc in Lewis rats with Streptococcal cell wall-induced arthritis. A single dose of tgAAV-ratTNFR:Fc administered via intra-articular injection resulted in suppression of arthritis in both the injected joint and the contralateral joint. The pharmacokinetics of intra-articularly injected tgAAC94 was tested in the non-human primate model. Expression of human TNFR:Fc was detected in joint lavage fluid of several animals injected with doses ranging from $1x10^{11}$ to $1x10^{13}$ DRP/mL of joint volume. In the rat model the biodistibution of tgAAV-ratTNFR:Fc to extra-articular tissues was transient and not detectable by day 90. There was no detectable distribution of the vector to the gonads. The safety of tgAAC94 over a range of doses (~1×10^{11} to 1×10^{13} DRP/mL of joint volume) was demonstrated in normal Lewis rats. No toxicity attributable to the test article was noted at the maximal dose that could be administered based on vector concentration and joint volume constraints. Similarly, no toxicity was noted in studies of tgAAV-ratTNFR:Fc in normal and arthritic rats. In nonclinical studies of tgAAV-ratTNFR:Fc and tgAAC94 after intra-articular injection very low levels of TNFR:Fc protein were detected in serum of only four animals (< 5% of tested). In these four animals the serum levels were <25 ng/mL which is at least two logs lower than the serum levels of human TNFR:Fc obtained during twice weekly treatment of human patients with 25 mg Enbrel. Together, the nonclinical data set demonstrates that local intra-articular delivery results in efficacy with lower systemic exposure to the secreted transgene and safety at doses at least one log higher than those expected to be therapeutic.

The first clinical trial of tgAAC94 will be conducted in subjects with RA who have persistent swelling in one or more joints despite a stable medical regimen for RA that includes at least one disease-modifying antirheumatic drug (DMARD) for the previous three months. The primary end point of this randomized, double-blind, placebo-controlled study is to evaluate the safety of intra-articular injections of tgAAC94. Four cohorts of eight subjects each will be enrolled and receive a single intra-articular injection of study drug, with six subjects in each cohort receiving tgAAC94 and two receiving placebo. The dose of tgAAC94 will be

increased between the first three cohorts. To expand the safety profile and gather additional information about efficacy, subjects will be enrolled in a fourth cohort and receive tgAAC94 at the highest dose determined to be safe after review of the safety data from the first three cohorts. Safety data will be reviewed after each cohort is enrolled, prior to enrolling the next cohort. Safety assessments will include clinical examination, laboratory monitoring and adverse event reporting. Secondary end points will include improvement in tenderness and swelling in the injected joint, improvement in tenderness and swelling in non-injected joints, and improvement in overall disease. The expression of tgAAC94 at injected site will be assessed by testing for humanTNFR:Fc protein in joint fluid. The development of serum neutralizing antibodies to AAV2, the presence of tgAAC94 in peripheral blood mononuclear cells and the amount of hTNFR:Fc protein in serum will also be determined.